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13. ABSTRACT (Maximum 200 words)  This was a prospective study with the goal of the effects of long-term supplementary feeding of glutamine versus a placebo on some aspects of immune cell function, on the incidence of infections and on mood. Highly trained US Air Force cadets, who were also elite runners, were selected for this study because their life style is generally well regulated, thus decreasing the possibility of extraneous variables. For all subjects the increase in white blood cells after VO <sub>2</sub> max tests, although much less marked, was similar to that normally observed after a marathon. An increase in IL-6 indicates inflammatory and immune response initiated by injury or possibly infection. A marked decrease in neutrophil activity was observed in these cross-country runners after VO <sub>2</sub> max tests, compared with that observed in other samples during training. There was a decrease in the IL-2 cytokine in the two T-lymphocyte subsets (T-helper cells - CD4; and T-cytotoxic cells - CD8) in the glutamine group compared with the placebo group in the sample taken halfway through the study.				
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## FINAL REPORT TO EOARD

### THE EFFECTS OF GLUTAMINE FEEDING UPON SOME ASPECTS OF THE IMMUNE SYSTEM OF AIRFORCE PERSONNEL UNDERGOING INTENSIVE TRAINING

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*In addition, three cadets helped with the study as part of their special Biology course research projects*

#### **Background to the project**

There is a high incidence of infection, particularly upper respiratory tract infection in endurance athletes undertaking prolonged, exhaustive exercise. This is more pronounced during the colder months, for example a 50% increase in infections has been observed in military personnel undertaking intensive training in winter. This may be due to immunodepression. There is increasing evidence that glutamine, which is an important fuel for some key cells of the immune system, is a conditionally essential amino acid which plays a major role in stressful conditions such as major surgery or prolonged, exhaustive exercise. The present investigation was a prospective study with the goal of the effects of long-term supplementary feeding of glutamine, versus a placebo (malto-dextrin) on some aspects of immune cell function, on the incidence of infections and on mood. Highly trained US Airforce cadets, who were also elite runners, were selected for this study because their life style is generally well regulated, thus decreasing the possibility of extraneous variables.

#### **Methods**

Twenty-seven cross-country runners (6F, 21M, aged 18-25) were recruited to an eight-week study (Jan-March 1998) investigating the effects on some aspects of the immune system of a winter training regime leading up to regional championships (WAC). At the end of this training period, the athletes were asked to participate in a bout of exhaustive exercise ( $VO_{2max}$

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tests). Ethical permission was obtained from the Surgeon General's office for the study. Fasting blood samples were taken several weeks before the Christmas break (S1) so that a "dry run" could be carried out to test the logistics of undertaking various aspects of the study. Fasting samples were also taken at the start of the study, immediately after the break (S2); halfway through the study (S3); at the end of the study, immediately after performing the  $VO_{2max}$  tests at 8 weeks (these samples were taken late morning/afternoon; S4); and the morning after these tests (S5). Measurements included numbers of circulating total white blood cells (WBC), neutrophils and lymphocytes; neutrophil activity, as measured by the oxidative burst technique, and some cytokines (CD4 and CD8 IL-2, IL-6, IL-8). In addition, Profile of Mood States (POMS) questionnaires and Cognitive Hardiness questionnaires were given to assess mood changes. Physical characteristics of the subjects were measured and the percentage body fat (Table 1) was estimated after the  $VO_{2max}$  tests, using the BodPod air displacement plethysmograph: this method compared well with the more traditional water displacement method. Heart rate was also monitored at this time.

**TABLE 1:** *Physical characteristics of subjects and  $VO_{2max}$  data (Means  $\pm$ SE)*

<i>Sex</i>	<i>Body wt (lbs)</i>	<i>% Body fat</i>	<i><math>VO_{2max}</math> (ml/kg/min)</i>
F	119.2 $\pm$ 2.9	17.88 $\pm$ 0.6	59.9 (range 56.9-62.3)
M	150.8 $\pm$ 2.5	8.15 $\pm$ 0.5	69.7 (range 62.4-81.7)

The cytokine analysis was carried out at David Grant Medical Center, Travis AF, using a new and interesting technique for measuring intracellular cytokines; whole blood cell counts and cortisol were measured at the USAF Academy Hospital; plasma glutamine and neutrophil activity were measured in Oxford, U.K.;  $VO_{2max}$  tests were undertaken and the data analysed in the Dept of Athletics, USAF Academy. POMS data was analysed by Dr Jon French, Brooks AFB; the Cognitive Hardiness data was undertaken and analysed by Captain Jon Drummond, USAF Academy Dept of Psychology.

*Statistical analysis:* The Principal Investigator (Linda Castell), together with Dr Mario Cortina at the Oxford University Department of Biostatistics, analysed all parameters using the

Linear Mixed Effects model (*Vonesh & Chinchilli, 1997, Linear and Nonlinear Models for the Analysis of Repeated Measurements. New York: Marcel Dekker Inc.*).

Due to injury etc., several subjects dropped out of the study after the start: thus most of the data was collected on nineteen subjects (4F, 15M). Questionnaires were given on the incidence of infection: unfortunately, the cadet helping with this part of the study found it difficult to "police" the return of these questionnaires. Compliance was disappointingly poor, to the extent that there was insufficient data to undertake statistical analysis on the infection questionnaires.

## RESULTS

*The effects of training and exhaustive exercise for all subjects, and for males vs. females:*

At S1 there was no difference in WBC or neutrophil numbers between males and female participants but there was a significantly higher number of lymphocytes in females than in males ( $p < 0.02$ ). At S2, neutrophil numbers were significantly higher in females than in males. No gender differences were observed at S3, or immediately after  $VO_{2max}$  (S4) but males had nearly two-fold higher circulating lymphocyte numbers than females the day after the  $VO_{2max}$  tests (S5;  $p < 0.01$ ).

For all subjects there was a marked increase in numbers of WBC ( $p < 0.006$ ) at S4 compared with the other samples. There was an increase in CD4IL-6 and CD8IL-6 in all subjects at S4 ( $p < 0.001$  and  $0.02$  respectively) (Table 2).

There was a marked reduction in neutrophil activity (75%;  $p < 0.001$ ) in all subjects immediately after  $VO_{2max}$  (at S4) compared with the other samples (Table 2).

*The effects of training and exhaustive exercise in the glutamine group vs. placebo group*

Most parameters showed no difference between the two groups. However, the following significant differences were observed:

*Circulating neutrophil numbers:* The placebo group had lower numbers of circulating neutrophils than the glutamine group at S3.

*Circulating lymphocytes numbers:* There were lower levels of peripheral blood lymphocytes in the glutamine group in samples S2 and S3 but no significant difference between the group was observed at S4 or S5.

*Neutrophil activity:* Neutrophil activity (measured as mean fluorescence per cell) was lower in the glutamine group than in the placebo group at S4.

*CD4-IL2 and CD8-IL2:* This cytokine was considerably lower in the glutamine group at S3 than in the placebo group for both T-cell subsets.

TABLE 2: *Whole blood cell numbers ( $1 \times 10^{-3}/\text{ml}$ ); CD4 and CD8 lymphocyte IL6; neutrophil activity (mean fluorescence per cell) in samples during training and before and after  $\text{VO}_{2\text{max}}$ . Statistical significance is denoted as: <sup>a</sup> $p < 0.001$ ; <sup>b</sup> $p < 0.02$ ; <sup>c</sup> $p < 0.05$ , compared with S2.*

	S2 (n=24)	S3 (n=18)	S4 (n=19)	S5 (n=18)
White blood cells	4.9 $\pm$ 0.3	5.5 $\pm$ 0.3	9.6 $\pm$ 0.6 <sup>a</sup>	5.9 $\pm$ 0.5
CD4-IL6	0.08 $\pm$ 0.01	0.67 $\pm$ 0.26 <sup>b</sup>	0.32 $\pm$ 0.06 <sup>a</sup>	0.14 $\pm$ 0.02
CD8-IL6	0.039 $\pm$ 0.006	0.11 $\pm$ 0.05	0.09 $\pm$ 0.02 <sup>c</sup>	0.11 $\pm$ 0.046
Neutrophil activity	62 $\pm$ 6	69 $\pm$ 6	15 $\pm$ 2 <sup>a</sup>	62 $\pm$ 7

## CONCLUSIONS

For all subjects the increase in white blood cells after  $\text{VO}_{2\text{max}}$  tests, although much less marked, was similar to that normally observed after a marathon. The increase in IL-6 indicates the inflammatory and immune response initiated by injury or possibly infection. A marked decrease in neutrophil activity was observed in these cross-country runners after  $\text{VO}_{2\text{max}}$  tests, compared with that observed in other samples during training. This is indicative of immunodepression, and is likely to be principally due to the additional bout of exhaustive exercise, although this was of relatively short duration (ca. 12min), after the regional championships at the end of eight weeks training. This emphasizes the importance of allowing adequate recovery time after a strenuous training programme.

The fact that there was no difference in plasma glutamine concentration between the two groups might indicate that 5g Gln per day was insufficient to have much impact on a daily variation in plasma glutamine. However, it should be borne in mind that glutamine feeding studies at rest have shown a marked increase in plasma glutamine 30-45 min after ingesting a 5g dose, returning to baseline levels after >2 hrs (Castell & Newsholme, 1997, *Nutrition*). In the present study all blood samples, except those after the  $\text{VO}_{2\text{max}}$  tests, were taken fasting, early a.m. Since the supplementary glutamine was consumed after training sessions, an increase in plasma glutamine would not be apparent in these blood samples taken the following morning.

There was a decrease in the IL-2 cytokine in the two T-lymphocyte subsets (T-helper cells - CD4; and T-cytotoxic cells - CD8) in the glutamine group compared with the placebo group in the sample taken halfway through the study. This correlates with the decrease in circulating total lymphocyte numbers observed at the same time point. Regrettably, circulating numbers of CD4 and CD8 cells were not measured because of financial constraints.

### **Future studies**

A larger study is planned, using higher doses of glutamine: 10g per day has already been given to elite rowers over several weeks without problems. Important points to address would be:

- (1) Ensuring that the questionnaires are properly administered and returned;
- (2) Including measurement of T-lymphocyte subsets, particularly CD4 and CD8;
- (3) Measuring an additional cytokine, viz. IL-8, which is a chemoattractant for neutrophils. This is particularly important in view of the marked differences observed in neutrophil activity in this study.
- (4) Dietary analysis for at least one week during the period of study.

It would also be of interest to look at the effects of altitude on those elite endurance athletes who come to the Academy from sea-level.

Some of the work has already been presented at the International Society of Exercise and Immunology meeting in Rome, and it is planned to present more data at another conference later this year. Other presentations/manuscripts which will arise from the study are listed below.



## **Publications arising from the project**

### *Abstracts*

Castell, L., Atchley, D., Bravo, N., Niemeyer, D., Reyes, A. & Bradshaw, P. (1999). Effect of eight weeks of training and exhaustive exercise on some aspects of the immune system. *Int. J. Sports Med.*, Proc. 4<sup>th</sup> Int. Symp. Exercise & Immunology, Rome. (In Press).

Castell, L.M. et al. (1999). The effect of glutamine vs. placebo upon cross-country runners undergoing intensive training for eight weeks. *Proc. Physiol. Soc.*

### *Full papers*

Castell, L.M., Atchley, D., Bravo, N., Niemeyer, D., Reyes, A. & Bradshaw, P. (1999). The effects of glutamine feeding upon some aspects of immune function in cross-country runners during training. (in preparation)

Atchley, D., Zupan, M., Bradshaw, P. & Castell, L.M. (1999). Maximal exercise testing in cross-country runners after a regime of 8 weeks training plus glutamine feeding. (in preparation)

Bradshaw, P., French, J., Drummond, J., Atchley, D. & Castell, L.M. (1999). Mood and cognitive hardness tests in athletes taking glutamine supplementation during training sessions. (in preparation).